STUDIES IN THE INTERMEDIARY METABOLISM OF AMINO ACIDS

BY

JULIUS EARL JOHNSON, JR.

A. B., University of Colorado, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1943

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STUDIES IN THE INTERMEDIARY METABOLISM OF AMINO ACIDS

PART I

THE METABOLISM OF VALINE IN PHLORHIZIN GLYCOSURIA*

INTRODUCTION

*cf. Rose, Johnson, and Haines.[1]

The phlorhizinized animal has been extensively employed by biochemists as an experimental subject for the study of metabolic reactions. Investigations of the animo acids have shown most of them to be precursors of either glucose or acetone-bodies in the diabetic dog. After a study of the fate of valine, Dakin[2] concluded that it was neither a glucose nor an acetone former. These results were unexpected in view of the fact that isobutyric acid[3], a proposed intermediate of valine catabolism, contributes three of its carbon atoms to the formation of glucose. It was desirable, therefore, to repeat the work of Dakin in an attempt to confirm or refute previous conclusions.

EXPERIMENTAL

Healthy female dogs which had borne pups were phlorhizinized. Complete twenty-four hour urine samples were obtained by catheterization. Analyses of the urine were made for glucose, total nitrogen, and acetone-bodies. In order to eliminate the possibility that renal retention had influenced the nitrogen excretion, the level of non-protein nitrogen in the blood was determined daily.

After the establishment of relatively constant G:N ratios, each for two to three days, the compounds to be studied were administered. It is highly important to the success of this type of experiment that all conditions be standardized as much as possible. A fixed routine should be maintained from day to day and from dog to dog. Establishment of reasonably constant G:N ratios is imperative. However, once a basic value has been attained, the substance to be tested should be administered without further delay.

Following the administration of *l*(+)-valine, *d*(—)-valine, and *dl*-valine extra glucose appeared in the urine in amounts which clearly demonstrated that three of the five carbon atoms of these compounds were employed in the production of sugar.

According to the generally accepted concept of amino acid catabolism the keto acid is the first product of oxidative deamination. Thus, sodium-α-keto-isovalerate was administered to phlorhizinized dogs. As was expected, glucose was found in amounts equivalent to three carbon atoms.

SUMMARY

In the phlorhizinized dog, *l*(+)-valine, *d*(—)-valine, *dl*-valine and sodium-α-ketoisovalerate contribute three of their five carbon atoms the formation of glucose.

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PART II

Study of the Ability of Alpha-Ketoisovaleric Acid and Alpha-Hydroxyisovaleric Acid to Replace Valine for Purposes of Growth in the Rat

INTRODUCTION

The existence of ketonic derivatives as normal intermediates in the metabolism of animo acids was first recognized through the classical experiments performed by Neubauer [1/2]. However it remained for Knoop[3,4] to prove definitely that certain keto acids are in reversible equilibrium with their corresponding amino compounds. Moreover, hydroxy acids are considered to be metabolic by-products which arise from the keto acid by a process of asymmetric reduction[5]. In view of the reversibility of many body reactions it might be expected that a dietary amino acid would be replaced by its keto or hydroxy analogue.

It is possible to use the method of growth as an index of the

effectiveness with which a compound may be substituted for an indispensable amino acid in the diet of the rat. Several keto and hydroxy acids have been tested and found capable of this replacement. Previous experiments by Rose and Eppstein[6] yielded inconclusive results as to the ability of valine to be replaced by its corresponding keto acid. The aim of the present investigation was to secure more definite evidence concerning this metabolic reaction. In addition, studies of the replacement of valine by *dl*-α-hydroxyisovaleric acid were repeated.

EXPERIMENTAL

Albino rats, twenty-nine days old, weighing 45 to 53 gm. received diets which were devoid of protein but contained a mixture of highly purified amino acids. The valine of the ration was replaced by the sodium salts of α-ketoisovaleric acid or α-hydroxyisovaleric acid. The growth rate of the animals was compared to that of positive controls receiving valine. Feeding experiments were conducted for a period of twenty-eight days.

The results clearly indicate that the rats which received either sodium-α-ketoisovalerate or sodium-α-hydroxyisovalerate in place of dietary valine grew approximately as well as the positive controls.

SUMMARY

By the method of growth as an index of physiological activity, it has been shown that sodium-α-ketoisovalerate is capable of replacing valine of the diet.

The ability of sodium-α-hydroxyisovalerate to replace the corresponding amino acid in the nutrition of the rat has been confirmed.

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PART III

Further Observations on a Unique Syndrome in Rats Deprived of Valine

INTRODUCTION

Previous experiments conducted by Rose, and Eppstein[1] have demonstrated the occurrence of a peculiar syndrome in rats which are fed diets devoid of valine. By the twenty-eighth day of deprivation the animals were sensitive to touch, lacked coordination of movement, and walked with a staggering gait. Moreover, they retracted their head and frequently displayed a rotatory motion. The symptoms were readily cured by the administration of valine without any other therapeutic measure.

It was desirable to repeat this work in an attempt to determine whether or not the syndrome could be consistently reproduced. In order to eliminate the possibility that a vitamin deficiency might have been involved, the control rats were fed diets which contained more complete vitamin supplements than those used in the earlier experiments. Furthermore, studies were conducted upon the effects of prolonged threonine and histidine deprivation. This was done so as to determine whether or not the symptoms observed were the result of valine deprivation specifically or the prolonged absence of any indispensable amino acid.

EXPERIMENTAL

Growth studies were performed upon albino rats weighing from 45 to 55 gm. Insofar as possible, proteins were completely absent from the diet. They were replaced by a mixture of amino acids shown to be analytically pure by a determination of their nitrogen content. Natural amino acids, obtained by isolation, were recrystallized three times after they yielded correct analytical values.

Successive groups of rats were then deprived of valine threonine, or histidine, and the behavior of the subjects was carefully observed.

From Dr. Emmett Holt[2] it was learned that valine deprivation

eventually causes a loss of the righting reflex in rats. Our results confirm this observation. After sixty days, the missing amino acid was returned to the diet. Thereafter, the ability of the rats to right themselves was tested twice daily. There was a definite restitution of the righting reflex after valine had been incorporated in the food for an interval of six to eleven days.

Beginning with the thirty-second day of valine deprivation the symptoms described by Rose and Eppstein[1] were becoming pronounced in all of the animals. On the other hand, the withdrawal of threoning and histidine from the ration for a period of forty days produced absolutely no visible manifestation of abnormal behavior comparable to those observed after an equal period of valine deprivation.

SUMMARY

It has been possible to confirm the observations of Rose and Eppstein that prolonged valine deprivation produces a striking and unique syndrome in rats. The absence of threonine or histidine for a period of forty days causes no symptoms similar to those observed in the study of valine deficiency.

The syndrome caused by the absence of valine is characteristic of this particular amino acid, and is not attributable to the mere absence of an essential component of the diet.

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PART IV

The Nutritive Significance of Certain Amino Acids in Man

Previous experiments[1] have shown ten of the twenty-two amino acids generally recognized as components of protein to be indispensable dietary constituents for animals. Of these acids, nine are essential for growth and even for maintenance. The tenth, arginine, is not necessary for fairly satisfactory growth but is required for maximum increase in weight.

The present investigation is a study of the amino acid requirements necessary for the maintenance of nitrogen equilibrium in the human species.

EXPERIMENTAL

Two healthy young men, weighing 59.6 and 63.7 kilos respectively, served as the subjects. The diet furnished 7.02 gm. of nitrogen daily of which more than 95 per cent was in the form of a mixture of the ten amino acids known to be indispensable for rats. The diets differed slightly with respect to their carbohydrate content, and yielded 2880 and 3060 calories per day respectively. The vitamins were supplied in daily doses of cod liver oil, and appropriate quantities of crystalline thiamine, riboflavin, pyridoxine hydrochloride, nicotinamide, ascorbic acid, calcium pantothenate, α-tocopherol, and 2-methyl-1,4-napthoquinone. The unidentified factors were obtained from a concentrate of liver equivalent to 5 gm. daily of Wilson's "Liver Concentrate Powder 1-20."

Approximate nitrogen equilibrium was effected in both subjects when the nitrogen intake was 7.02 gm. daily, and persisted throughout the fore period of eight days. At the expiration of the fore period, valine was removed from the food and the other amino acids were increased sufficiently to provide a constant nitrogen intake. The effects of this change were profound. Immediately, each subject manifested a negative nitrogen balance. On the fourth day the nitrogen output exceeded the intake by 2.19 and 2.91 gm. respectively. Valine was then returned to the food and was followed quickly by the reestablishment of positive nitrogen balances.

After six days upon the complete diet, methionine was excluded from the food for a period of five and six days respectively. Again, the subjects lost more nitrogen than was ingested, although the negative balances (0.51 and 1.60 gm.) were not so large as after valine deprivation. Immediately after the administration of methionine, positive balances ensued.

SUMMARY

The fact that equilibrium can be maintained upon a diet containing only ten amino acids demonstrates that the amino acids

previously shown to be dispensable for rats and dogs are also dispensable for man.

Since the omission of valine or methionine causes the onset of negative balances, these amino acids are undoubtedly indispensable constituents of the human diet.

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VITA

The author was born April 10, 1917, in Denver, Colorado. He received his elementary education in Littleton, Colorado, and his secondary education in the Public Schools of Denver. He attended the University of Denver from 1935 until 1937. He then transferred to the University of Colorado, from which he received his Bachelors Degree in Chemistry in June, 1939. He entered the graduate school of the University of Illinois in the Fall of 1939, and has held a Special Research Assistantship in Chemistry since that time.